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Computation of Bhat's OMIT maps with different coefficients

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Abstract

The OMIT electron-density-map calculation of Bhat [Bhat & Cohen (1984). *J. Appl. Cryst.* **17**, 244–248; Bhat (1988). *J. Appl. Cryst.* **21**, 279–281] is very effective in discovering errors in a macromolecular structure determination. A Fortran program called *OMIT* has been written to calculate such maps and an investigation has been carried out into which coefficients for the map calculation produce the best OMIT maps. Testing of the program on Savinase™ showed that the best overall results were obtained when $|F_o|$ without figure of merit was used. In regions where the map is incorrect, the most interesting OMIT maps are produced when only the figure of merit, or modified *SIGMAA* coefficients, are used as the initial map amplitude coefficients. Thus, these tests suggest that such OMIT maps are particularly useful to reconstruct the macromolecular model in the grossly incorrect regions of the model.

1. Introduction

The OMIT electron-density maps computed using Bhat's procedure (Bhat & Cohen, 1984; Bhat, 1988) are known to be very effective for the discovery of errors in the crystal structure determination of macromolecules. However, in their publications, Bhat and coauthor have not investigated the effects of different Fourier coefficients on the quality of the resulting OMIT maps. The subject of this paper is the study of several types of amplitude coefficients for the computation of the initial electron-density distribution from which OMIT maps are calculated, and the analysis of the resulting maps.

2. Definitions

$|F_o|$ and $|F_c|$ are the observed and calculated structure-factor amplitudes, respectively, and $|E_o|$ is the normalized structure-factor amplitude for the observed reflection. m is Sim's figure of merit. $m = I_1(X)/I_0(X)$ for acentric reflections and $m = \tanh(X/2)$, where I_1 and I_0 are the modified Bessel functions of order 1 and 0, respectively, and $X = 2|F_o||F_c|/\Sigma_L$ ($\Sigma_L = \sum F_i^2$).

SIGMAA: Read (1986) has shown that electron-density maps calculated with amplitude coefficients of the form $2m|F_o| - D|F_c|$ for acentric reflections and $m|F_o|$ for centric reflections are less biased towards the macromolecular model from which the $|F_c|$'s are calculated. In this work, we have used modified *SIGMAA* coefficients of the form $3m|F_o| - 2D|F_c|$ for acentric reflections and $2m|F_o| - D|F_c|$ for centric reflections (*vide infra*).

OMIT maps: the reader is referred to Bhat (1988) for a complete description of the reciprocal-space procedure used to compute such maps, in which the electron density of a 'phasing volume' plus that of a 'neutral volume' surrounding it do not contribute to the $|F_c|$'s and ψ_c's used in the procedure.

CCF1 is a real-space correlation coefficient with respect to a 'control map', without any resolution cut-off, computed from refined atomic coordinates. CCF2 is a correlation coefficient between the OMIT map and a 2.0 Å-resolution artificial electron-density distribution generated from refined atomic coordinates.

3. Material and methods

A Fortran program called *OMIT* was written that allows one to compute Bhat's OMIT maps in a single step. This program requires amplitudes and phases in the *CCP4* (Collaborative Computational Project, Number 4, 1994) MTZ format, and generates an OMIT map in the Groningen *BIOMOL* Master Fourier Map (MFF) format, which can be converted to other formats (*e.g. DSN6*, *CCP4*, *X-PLOR*) using separate programs that are available from the authors.

In this study, we have used 'classical' coefficients of the form E_o, F_o, mF_o, $2F_o - F_c$ *etc.* (Table 1) to compute the initial electron-density distribution from which Bhat's OMIT maps are calculated. Concomitantly, we have developed a new type of computation of OMIT maps: in the first step, a 'classical' electron-density map is computed and the histogram of densities (Lunin, 1988; Zhang & Main, 1990) of this map is generated. The OMIT map is then computed and, finally, the histogram of densities of the OMIT map is adjusted to fit that of the initial electron-density map.

All our tests have been carried out using models of the structure of monoclinic Savinase™ in complex with the active site inhibitor benzo-furan boronic acid ($BC_8O_3H_7$) (Verger, Vellieux, Kiersten Nielsen & Fontecilla-Camps, 1997). We used the 2.0 Å-resolution unrefined model for the computation of the calculated amplitudes and phases used in the OMIT-map calculations. For the computation of the real-space correlation coefficient CCF1 between the observed OMIT density and the calculated density generated from the refined molecular model, carried out with the program *O* (Jones, Zou, Cowan & Kjeldgaard, 1991), the 1.7 Å-resolution refined model of Savinase™ was used to generate a calculated density with no resolution cut-off. We also computed, using the program *MAPMAN* (Kleywegt & Jones, 1996), the correlation coefficient CCF2 between the OMIT maps and a 2.0 Å-resolution artificial electron-density distribution generated from refined atomic coordinates.

Savinase™ crystallizes in space group $P21$, with cell dimensions $a = 40.83$, $b = 72.91$ and $c = 76.27$ Å and $\alpha = 90.0$, $\beta = 91.21$ amd $\gamma = 90.0°$. The crystals contain two molecules of Savinase™ in the asymmetric unit. For the OMIT-map calculations, the asymmetric unit was divided into 30 phased volumes, each surrounded by a neutral volume

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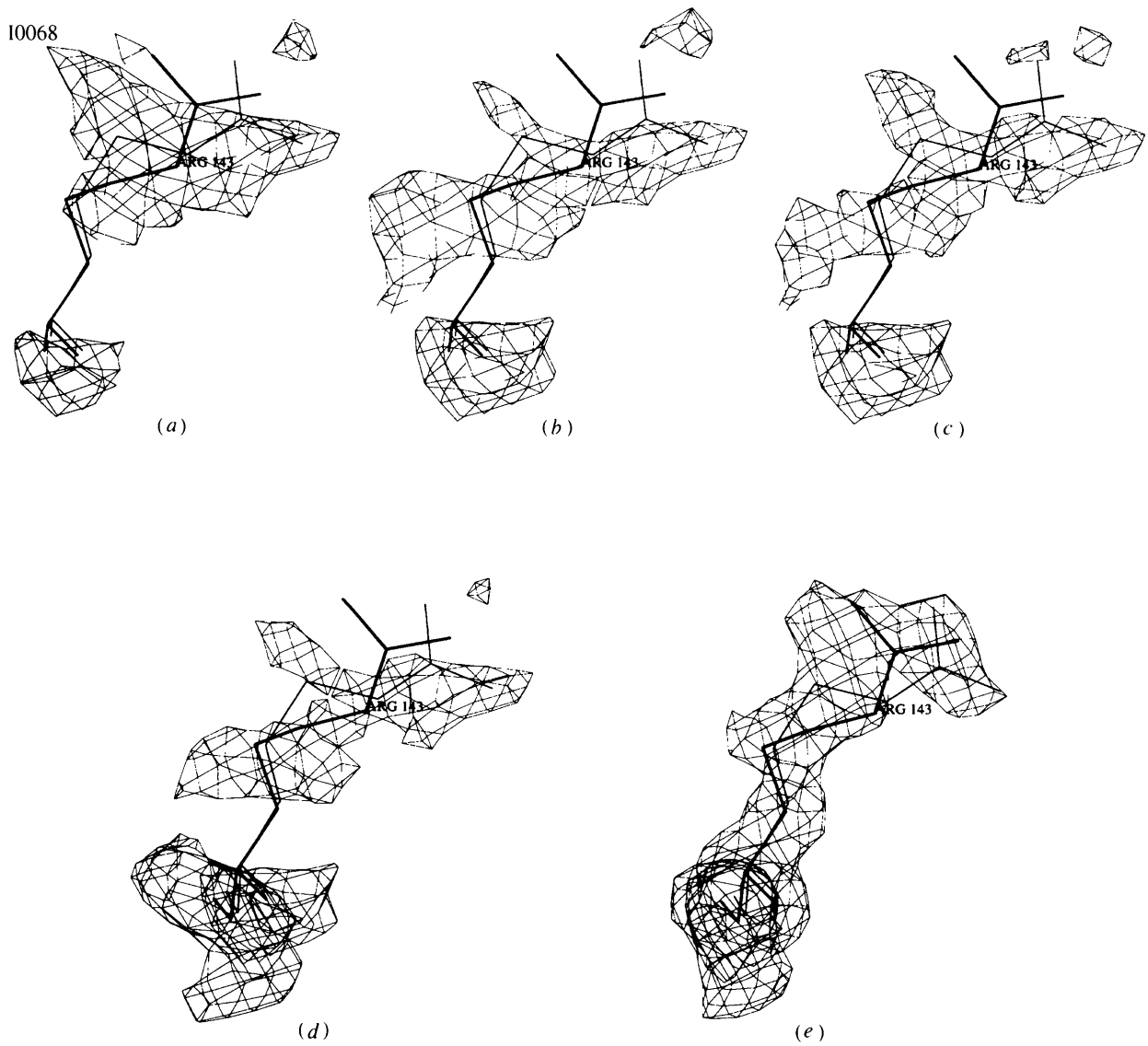

Fig. 1. OMIT electron-density maps, computed with different initial amplitude coefficients. Contrary to the electron densities usually shown, this figure displays the OMIT electron density for Arg143, which is the most difficult region of the map to interpret: at 2.0 Å resolution, there is evidence for a double conformation for this side chain; at 1.7 Å resolution, from new data collected from a new crystal, the OMIT electron density suggests the presence of only one conformer for this side chain. Note that refinement displaced the Arginine side chain from one of the two conformations to its correct single conformation, located midway between the two conformations observed at 2.0 Å resolution for this side chain. (*a*) 2.0 Å-resolution OMIT map computed with initial amplitude coefficients of the form $3|F_o| - 2|F_c|$ and phases from the unrefined model of Savinase™. (*b*) As (*a*), with initial amplitude coefficients of the form $|F_o|$. (*c*) As (*a*), with initial amplitude coefficients of the form m. (*d*) As (*a*), with modified *SIGMAA* initial amplitude coefficients of the form $3m|F_o| - D|F_c|$ for the acentric reflections. (*e*) 1.7 Å-resolution OMIT map computed with modified *SIGMAA* initial amplitude coefficients and phases from the refined model of Savinase™. This map was computed using the prerelease of *X-PLOR* Version 4.0 (Brünger, personal communication; Brünger, Kuriyan & Karplus, 1987). Thin lines: 2.0 Å-resolution model of Savinase™ before refinement; thick lines: 1.7 Å-resolution model of Savinase™ after refinement with *X-PLOR*. All maps were displayed using the program *O* (Jones, Zou, Cowan & Kjeldgaard, 1991) and are contoured at the 1.0σ level.

Table 1. *Summary of the results obtained with several amplitude coefficients for the computation of the initial electron-density distribution from which the OMIT map is calculated*

HM: mapping of the histogram of densities of the OMIT map to that of the initial map. m: figures of merit computed according to Sim (1959, 1960), except for the modified *SIGMAA* coefficients (m), where they are computed according to Read (1986). Min CCF1: minimum value of the real-space correlation coefficient, calculated with the program *O* (Jones, Zou, Cowan & Kjeldgaard, 1991) between the OMIT map and the artificial electron-density distribution obtained from refined atomic coordinates, without any resolution cut-off, for one of the molecules in the asymmetric unit on a residue-per-residue basis. Max CCF1: maximum value of the real-space correlation coefficient. ⟨CCF1⟩: average value of the real-space correlation coefficient. CCF2: correlation coefficient computed using the program *MAPMAN* (Kleywegt & Jones, 1996) between the OMIT map and the 2.0 Å-resolution artificial electron-density distribution computed from the refined molecular model. The maximum values of the correlation coefficients are shown in **bold**.

	With HM			Without HM			
Coefficient	Min CCF1	Max CCF1	⟨CCF1⟩	Min CCF1	Max CCF1	⟨CCF1⟩	CCF2
$\|E_o\|$	0.390	0.852	0.659	0.390	0.849	0.663	0.478
$\|F_o\|$	0.380	0.856	0.663	0.396	0.853	**0.669**	**0.479**
$m\|F_o\|$	0.348	0.844	0.643	0.348	0.844	0.644	0.454
$2\|F_o\| - \|F_c\|$	0.351	0.859	0.652	0.351	0.858	0.651	0.273
$m(2\|F_o\| - \|F_c\|)$	0.328	0.850	0.637	0.329	0.850	0.637	0.439
$3\|F_o\| - 2\|F_c\|$	0.252	0.837	0.605	0.251	0.838	0.604	0.395
$m(3\|F_o\| - 2\|F_c\|)$	0.255	0.841	0.622	0.257	0.843	0.621	0.416
$3m\|F_o\| - 2D\|F_c\|$	0.349	0.862	0.644	0.332	**0.864**	0.638	0.449
m	0.413	0.827	0.653	**0.416**	0.826	0.657	0.469

consisting of two grid points in each direction (terms are defined as in Bhat, 1988). Since simulated-annealing OMIT-map calculations (Hodel, Kim & Brünger, 1992) took exceedingly long to compute (so long that such a computation was not completed after more than 24 h CPU time on our three-processor DEC ALPHA server), we did not investigate the effect of such refinement procedures together with different initial amplitude coefficients on the quality of the resulting OMIT maps. These OMIT maps, including the step of histogram mapping, may now be computed using the prerelease of *X-PLOR* Version 4.0 (Brünger, personal communication; Hodel, Kim & Brünger, 1992; Brünger, Kuriyan & Karplus, 1987).

4. Results

Our results (Table 1 and Fig. 1) can be summarized as follows:

(i) Resetting the electron density values less than -1.0 times the root-mean-square (r.m.s.) electron density to -1.0 times the r.m.s. electron density, as suggested by Bhat (1988), gave poorer OMIT maps than those computed with omission of this step (data not shown). Hence, in all our tests, this truncation step was omitted, so that $|F_m| = |F_c|$, $W_f = 1.0$ and $W = W_\varphi$ for all reflections (these terms are defined as in Bhat, 1988). Attempts have not been made to vary the threshold or the resolution of the data to analyse the usefulness of non-negativity criteria in OMIT maps.

(ii) The highest correlations between the OMIT map and the calculated maps from the refined molecular model are obtained when no figure-of-merit (Sim, 1959, 1960) scheme is used, except for the OMIT maps computed with initial $3|F_o| - 2|F_c|$ amplitude coefficients and for modified *SIGMAA* initial amplitude coefficients of the form $3m|F_o| - 2D|F_c|$ for acentric reflections and $2m|F_o| - D|F_c|$ for centric reflections (the modified *SIGMAA* coefficients give improved electron-density maps with respect to the 'classical' coefficients of the form $2m|F_o| - D|F_c|$ for acentric reflections and $m|F_o|$ for centric reflections: because the crystallographic refinement problem is undetermined for protein structures, where we are usually far from atomic resolution, it is possible to overfit the amplitudes in structure refinement. The amplitude differences are therefore smaller than they would be if the errors in the structure were truly random. To correct for that effect, in part, we can scale up the contribution of the differences in the electron-density-map coefficients (Vellieux, unpublished work; Read, 1986; Vijayan, 1980).

(iii) The effect of adjusting the histogram of densities of the OMIT map to fit that of the initial map is negligible. This is perhaps not surprising *a posteriori*, since the application of histogram modification is important only for the real-space correlation-coefficient calculation and not for the map quality, since it is simply a nonlinear rescaling of the map and influences only the choice of the isoline level.

(iv) The most surprising result has been that, in the regions of the map where the model is incorrect, with a low real-space correlation coefficient, very interesting OMIT maps (minimum correlation 0.416) are obtained when only the Sim figure-of-merit is used as the initial map amplitude coefficient (Fig. 1*c*). This indicates, once again, that phases are more important than structure-factor amplitudes when it comes to the calculation of an electron-density distribution. Thus, these tests suggest that this type of OMIT map, together with OMIT maps computed with $|F_o|$ and with modified *SIGMAA* coefficients as the initial amplitude, are particularly useful to reconstruct the macromolecular model in the grossly incorrect regions of the model.

It has not escaped our attention that such OMIT maps may also be used as the starting point for electron-density modification procedures. Tests are currently being carried out using the *DEMON/ANGEL* density-modification software suite (Vellieux, Hunt, Roy & Read, 1995) to investigate such OMIT maps in this context. It can also be suggested, from the results summarized in Table 1, that the use of several OMIT maps, computed with different initial amplitude coefficients (*i.e.* only the Sim figure of merit m, $|F_o|$ and modified *SIGMAA* coefficients of the form $3m|F_o| - 2D|F_c|$ for acentric reflections and $2m|F_o| - D|F_c|$ for centric reflections) may be an optimal strategy in the detection of errors in a macromolecular model.

The *OMIT* program is available freely upon request to the authors (e-mail vellieux@ibs.ibs.fr or bauke@chem.rug.nl).

The members of the Institut de Biologie Structurale Jean-Pierre Ebel CEA CNRS dedicate this paper to the memory of Rosaria and Serge Pares, who died on board TWA flight 800 off the Long Island coast. We thank Professor A. T. Brünger (Yale University) for having provided the prerelease of *X-PLOR* Version 4.0 and Dr M. Frey (IBS/LCCP) for his critical reading of the manuscript. Financial support of the French CEA and CNRS, and of the Netherlands Foundation for Chemical Research (SON), is gratefully acknowledged.

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